Fig. VI. *Cerato-Lejeunea mauritiana* St. a. pars caulis a ventre visa. b. Perianthium.

Fig. VII. *Lopho-Lejeunea multilacera* St. Pars caulis c. per a ventre visa.

Fig. VIII. *Acro-Lejeunea parviloba* St. a. pars caulis a ventre visa. b. Folia involucr. c. amph.

Fig. IX. *Cerato Lejeunea Renauldii* St. a. pars caulis a ventre visa. b. perianthium.

Fig. X. *Lophocolea borbonica* St. a. pars caulis a ventre visa. b. perianthium. c. folium invol. d. amph. invol.

Fig. XI. *Lophocolea inflata* St. Pars caulis a ventre visa.

Fig XII. *Lophocolea longifolia* St. a. pars caulis c. flore fem. b. perianthium. c. Folia invol d. Amph. invol.

Fig. XIII. *Lophocolea rubescens* St. a. pars caulis a ventre visa. b. perianthium. c. Folia et amph. invol.

Fig. XIV. *Plagiochila Cambuena* St. a. folium caulinum. b. Amphigastrium.

Fig. XV. *Plagiochila Rodriguezii* St. Pars caulis a ventre visa.

Fig. XVI. *Plagiochila tenax* St. a. pars caulis a ventre visa. b. perianthium junius. c. folium florale.

Fig. XVII. *Schistocheila borbonica* St. a. Folium caulinum. b. apex plantae c. per.

Fig. XVIII. *Schistocheila piligera* St. Folium caulinum.

Celloidin imbedding in plant histology.

A. C. EYCLESHYMER.

Celloidin[1] imbedding is being used quite extensively in animal histology and offers many advantages over paraffine. It is with the view of introducing the methods into plant histology that I write the following, for which I claim but little originality.

Those who have imbedded delicate vegetable tissues, by methods requiring heat, are well aware of the extreme care necessary to avoid contraction. This is entirely avoided by the use of celloidin; moreover, alcoholic material may be imbedded directly.

To obtain the best results a celloidin free from all trace of foreign matter should be used. An excellent article is manufactured by the Chemische Fabrik auf Actien (E. Schering), Berlin. It may be obtained through the Ed. Supply Co., 6 Hamilton Place, Boston, or Bachrach & Brothers, Balti-

[1] Duval (Jour. de Microg., p. 226, 1888) states that celloidin has no advantages over collodion.

more. The prepared plates, or fragments, should be transparent, of a light yellow color, very tough and elastic; if brittle and possessing a milky opaqueness, it is of an inferior grade and the imbedding mass will be too brittle or soft.

The fragments are enclosed in an air tight chamber; a four oz. salt-mouth bottle answers well. Pour into this bottle just enough ether-alcohol (two parts sulphuric ether; one part absolute alcohol) to cover the fragments. I find that these proportions give better results than equal parts. The solution is easier and the hardening more rapid. The ether-alcohol should be added until, after occasional shaking and stirring, no fragments remain undissolved. This may take several days. It should finally possess the consistency of a very thick oil. The solution thus obtained may be labeled no. 4. No. 3 is obtained by taking two volumes of no. 4 and diluting with one volume of ether-alcohol. No. 2 by proceeding in a like manner with no. 3. No. 1 is a mixture of 95 % alcohol and sulphuric ether equal parts.[2]

The saturation and final imbedding is accomplished thus: The object is transferred from 95 % alcohol to solutions 1, 2, 3, 4, successively, in each of which it remains from a few hours to days, depending upon the size and permeability. For most tissues twenty-four hours in each will suffice. It often occurs that one desires merely to hold the object in situ for cutting; this is generally attained by passing the object through solutions 2 and 4.

In imbedding, the first thing necessary is to provide boxes. They may be made in the following manner:[3] The end of a pine block is trimmed to the desired size, e. g., 1 cm. long by 1.5 cm. wide. For a box of these dimensions, and 1 cm. deep, a piece of ordinary porous letter-paper may be cut in rectangular form 3 cm. wide by 6 cm. long.

Place the center of the end of the block over the center of the paper, the longer axes of the end of the block and paper parallel. The sides are now pressed against the sides of the block. Then the ends in the same way, leaving four projecting wings at the angles of the block. Fold in the wings against the narrower sides and press the ends of the paper, that now project above the level of the box, down over the

[2]Schiefferdecker (Arch. f. Anat. u. Phys., I Abth., p. 199, 1882) uses two solutions, one syrupy, the other much thinner. Objects are transferred from absolute alcohol to thinner and then to thicker. Minot (Whitman's Embryological Methods, p. 114, 1885) uses three solutions: 1. Ether and alcohol equal parts. 2. Thin solution of celloidin. 3. Thick solution of celloidin. I prefer the four solutions given by Apathy (Zeit. f. wiss. Mikr., p. 45–49, 1888.)

[3]Whitman's Embryological Methods, p. 97, 1885.

ends of the box. A thin plate of lead is placed in the bottom[4] and the imbedding solution poured in. The object is taken from the same solution and, with needles wetted in ether, placed in the desired position. Fine needles may be passed through the box to support the object. There are many other methods of imbedding.[5] I believe the method given, however, to be the best suited for general purposes.

Hardening is accomplished by various methods[5]. I prefer that given by Viallam,[6] of immersing in chloroform since the operations may be carried on with much greater rapidity. An air tight chamber should be filled with chloroform; a very wide-mouth bottle will answer. After it is thoroughly hardened, which requires about twenty-four hours, the mass is removed and the paper cut from the sides.

It is now ready to fix for sectioning. Basswood blocks are trimmed to fit the clamp of the microtome. Solution no. 3 is poured over the block and allowed to partially harden; pour on a little more of the same solution and press down the celloidin block, after dipping the under surface in ether, into it. Place in chloroform until hardened.

The sections are now cut. Care should be taken that the knife is placed as obliquely as possible and constantly wetted with 70 % alcohol. An ordinary wash-bottle may be used for this purpose, or one can easily arrange a dripping apparatus.[7] The sections may be removed, with a camel's hair brush, to a watch crystal containing an alcoholic stain. If an aqueous stain, they are passed through the lower grades of alcohol, 50 %, 30 %, to water, stained, run back through the

[4] Suggested by Prof. J. E. Reighard.

F. Blochmann ("Ueber Einbettungsmethoden," Zeit. f. wiss. Mikr., I, p. 218, 1884) prefers imbedding on cork. One end of a cork is surrounded by a strip of paper, which is fastened by a pin. In the cup thus formed the object is imbedded. A pin is passed through a piece of lead and stuck into the bottom of the cork. The mass is now placed in the hardening fluid. This method offers advantages, since hardening and fixing are obtained at the same time. Instead of cork, which gets soft and spoils the alcohol, because of the tannic acid, I use basswood cylinders, which are preferable to anything I have tried. Apathy (Zeit. f. wiss. Mikr., VI., pp. 164–70, 1889) uses glass boxes. Into these is poured the imbedding solution. The box is filled to the brim and covered with a glass plate. This prevents the surface from hardening and allows the air bubbles to escape. The plate is replaced in a few hours by a belljar, and when a film has formed, in six to eight hours, 75 % alcohol is poured on. In twenty-four hours the mass is ready for cutting. Flormann (Jour. Roy. Microscopical Soc., Feb., 1890) imbeds in a glass capsule in a thin solution, and solidifies by allowing the slow evaporation of the solvent. Thoma (Jour. Roy. Microscopical Soc., p. 305, 1883) covers a cork with a thick solution of celloidin. Upon this is placed the object which is covered, layer after layer, with celloidin, allowing each to partially dry. When the object is covered it is immersed in alcohol until dry.

[6] "Recherches sur l'Hist. des Insectes," Paris, 1883, quoted in Lee's Vade Mecum.

[7] See Whitman's Embryological Methods, p. 115.

grades: 30 %, 50 %, 70 %, 82 %, 95 %. If balsam mounts are desired they are transferred to absolute alcohol, cleared[8] and mounted.

I can highly recommend the use of phenol as a clearing medium, since it clears after low grades of alcohol. Dr. Bergonzini[9] transfers sections from aqueous stains directly to phenol which is followed by balsam. I have used a mixture of bergamot oil and phenol equal parts with excellent results. Creosote and oil of cloves dissolve celloidin but clear well. The sections may be transferred to a watch crystal filled with the clearing medium. After clearing they are arranged on the slide and the balsam applied.

If the object can be stained *in toto*, which is often the case, much time may be saved by the following method: The stained object is imbedded in the usual manner, but after hardening in chloroform, and removing the paper, the celloidin block is transferred to 95 % alcohol for twenty-four hours then to carbolic acid, bergamot oil or glycerine in which it becomes as transparent as glass.[10] The block is fixed in the usual manner.

Orienting is now accomplished with the greatest ease. In cutting, the knife is wet with the clearing medium. The sections may be arranged in serial order on the knife blade until a slide full is obtained, when they are transferred, balsam applied, and mounted. By this method long series[11] are handled with ease. Glycerine is used only when the mounting medium is glycerine or glycerine-jelly. Since these are used quite extensively the method may prove to be of value.

The blocks may be preserved for an indefinite time in alcohol, bergamot oil, carbolic acid, glycerine, etc. It is often desirable to mark the blocks for future reference. The

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[8] Weigert (Zeit. f. wiss. Mikr., p. 430, 1886) uses a mixture of three parts xylol and one part phenol for clearing sections stained with hæmatoxylin or carmine. Aniline stains are decolorized by its use. Unna (Jour. Roy. Microscopical Soc., p. 518, 1887,) states that glycerine and carbolic acid quickly and permanently extract all basic aniline dyes. Martinotti (Zeit. f. wiss. Mikr., p. 153, 1887) cautions against the use of xylol since it destroys delicate structures, such as karyokinetic figures. J. Van Gieson (Am Month. Micro. Jour., p. 49-51, 1887) claims that the only satisfactory clarifier is oil of Origanum.

[9] Lo Spallanzoni, p. 196, 1883.

[10] The method of clearing in mass is advised by Selenka (Zool. Anz., p. 130, 1878) for albumen.

[11] H. E. Simmons (Microscope, p. 73, 1887) gives a method of fixing sections in serial order. They are cut and arranged on the slide in the desired position. 95 % alcohol is applied for a few minutes then drawn off. Sulphuric ether vapor is poured over the sections in which they immediately soften. They are now transferred to 80 % alcohol which hardens the celloidin, retaining them in position, when they are run through the grades of alcohol, stained, dehydrated, cleared and mounted.

end of the block is wet in solution no. 1, a piece of paper with the desired data is stuck on and washed over with solution no. 3.

In conclusion I may say that I have tried nearly all the methods employed in celloidin manipulation and have succeeded best with the above, which is largely a combination or modification of methods already known.

Botanical Laboratory, University of Michigan.

The Collodion method in botany.

M. B. THOMAS.

In any original investigation in the field of histological botany the worker is at the outset confronted with the all important question of how to prepare the material so that it may be available in the best possible condition for thorough systematic observation and study. The old method of making freehand sections in careful investigations is now gone by and the best histologists look for some substance with which to infiltrate the tissue and bring it into a condition where uniform serial sections can be made. Many substances have from time to time been offered and met with varying success. Some of the more important ones that have been tried are gelatin, gelatin soap, gum, paper, shellac, wax, gum arabic, soap, paraffine and collodion. The last two substances have seemingly supplanted the others and indeed they seem to offer all the advantages that can be secured by any of the others.

The paraffine method as applied to plant tissue was published by Dr. Moll in the BOTANICAL GAZETTE of January, 1888, and later many modifications of it have been given and the method much improved. The method has been quite extensively used, but is very long and quite disagreeable to manipulate, often requiring 10 or 12 days to bring the tissue into proper condition for sectioning. For proper infiltrating with paraffine a temperature of from 45° to 50° C. is required, and for some of the more delicate tissues this in my hands has proved fatal. The method is admirably adapted, however, for many tissues that can be held in position for sectioning in no other way, as is the case with mature seeds or woody stems. The collodion method is now coming into general use for nearly all kinds of plant tissue. For the use